COUPLED LC-GC TECHNIQUES FOR THE CHARACTERISATION OF PAC IN FUEL AND ENVIRONMENTAL SAMPLES

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INTRODUCTION

Exposure to polycyclic aromatic hydrocarbons has long been identified as of considerable environmental concern[1]. Originating from both natural and anthropogenic sources, many PAC exhibit significant carcinogenic and mutagenic[2,3] properties which are critically dependent on structure. Although PAC occur naturally in fossil fuels, the predominant contributions to environmental pollution are caused by the combustion of organic fuels. Recent studies have identified characteristic source fingerprints for coke ovens, diesel and gasoline engines, road tunnels and wood combustion emissions[4]. However, despite the wealth of literature on the identification of parent polycyclic aromatic hydrocarbons[5,6] and polycyclic aromatic heterocycles[7,8], little is known about the composition and concentration of the many substituted and heteroatom-containing PAC that are produced either by combustion processes or as products of atmospheric reactions[9] and are potentially more of a health risk than parent PAH or are simply unknown[3,10]. Due to the complexity of fuel and environmental samples which contain many hundreds of aliphatic, aromatic and polar compounds, multidimensional chromatographic methods which provide separation by virtue of chemical class (group-type) or by molecular mass can greatly simplify the identification of individual PAC. LC-GC has been used previously for quantitative analysis of PAH in diesel exhaust emission extracts[11,12] urban air particulates[13,14] and other fuel related applications[15]. In this study, on-line coupled LC-GC techniques have been investigated for the identification of trace level PAC in a range of fuel feedstocks, combustion products and urban air particulate extracts. Positive identification of individual PAC was obtained by direct coupling of LC-GC to mass spectrometry and atomic emission detection.

EXPERIMENTAL

Samples: An urban air particulate sample was collected by high volume air sampler (Graseby Anderson, UK) drawing air at a rate of 0.4m^3 through a quartz microfibre filter (Whatman UK), using a previously described method[14]. The filter was placed in an extraction vessel at 110°C and was extracted with CO_2 and 10% toluene at 400 atm, for 90 minutes using an ISCO SFX 2-10 extractor with Model 100D and 260D syringe pumps. Pressure was maintained within the cell using a 25 mm i.d. x 10 cm length of fused silica capillary tubing. The extract was collected in 3 ml of dichloromethane, which was subsequently reduced to dryness and re-dissolved in *ca.* 2 ml *n*-hexane, for compatibility with the subsequent normal phase LC step. Previous extractions of standard dust samples (NIST SRM-1649) have showed that Soxhlet equivalent or better recoveries were obtained for all certified PAH; the results have been reported earlier[14]. A typical UK diesel sample was used for a preliminary investiagtion by LC-GC-AED.

LC-GC-MS Analysis: A Carlo-Erba Dualchrom 3000 Series LC-GC instrument (Fisons, Italy), consisting of two HPLC syringe pumps, a UV detector, on-column interface, early solvent vapour exit and GC was coupled via a heated transfer line to a Finnigan Mat ion trap detector mass spectrometer (ITD/MS)[10,13]. HPLC separation was performed using a 100 mm x 2 mm i.d. column containing 5 µm 'Spherisorb' silica (Phase Separations, UK) packing. Pentane and dichloromethane were used as the mobile phase, with a stepped polarity gradient. Sample injection was via a 20 µl loop injector. LC separation was monitored by UV detection (254 nm), and was recorded by chart recorder. From the detector the eluent passed into the on-column interface which in stand-by position routed the flow through to waste. On transfer a pneumatically actuated valve was used to switch the flow through a fused silica capillary column which transfers the sample onto the retaining pre-column. GC separation was performed on a 25 m x 0.32 mm i.d. fused silica capillary column coated with a 0.33 µm film of dimethyl siloxane (BPX-5 SGE, UK). The column inlet was connected by means of a press-fit connector to a 4 m section of the separation column which was used as a retaining pre column. The pre-column was connected to 10 m x 0.53 mm i.d. uncoated fused silica retention gap, deactivated by phenyldimethyl silylation. This connection also includes an early solvent vapour exit for the evaporation of mobile phase. The exit has a twofold advantage; firstly reducing the time required

to evaporate the mobile phase and, more importantly minimising the amount of mobile phase entering the detector(s). The partially concurrent solvent evaporation rate at 65°C for a 50:50 *n*-pentane : dichloromethane (%v/v) mobile phase using the solvent vapour exit was determined to be 100 µL/min at an inlet carrier gas pressure of 120 kPa. With an LC solvent flow rate of 100 µL/min, the helium carrier gas was essentially free of solvent contamination prior to the ion trap detector (ITD). On completion of the sample transfer via on column interface, the fused silica capillary column was removed from the GC inlet, to eliminate possible vaporisation of the polyamide coating and subsequent bleed into the carrier gas at high oven temperatures. Transfer of eluent from the GC oven to the ITD/MS for detection was via a heated transfer line at 250°C. Mass scans were performed at 1 scan/sec with a mass range of 50-350 amu.

LC-GC-AED Analysis: Normal phase HPLC was performed using an in-house built LC system[12]; a Brownlee Micro gradient dual syringe HPLC pump (Brownlee Labs, Santa Clara, USA), a Rheodyne 7040 injection valve (Berkley, USA) with a 20 ml sample loop and a 100 mm x 2 mm i.d. column packed with 5mm 'Spherisorb' silica (Phase Separations, UK). Detection was by means of a Uvikon 735LC UV detector (Kontron Instruments, UK) equipped with a 1 ml flow cell and operated at a wavelength of 254 nm. Eluate passes from the UV detector entering a ten-port valve fitted with a pneumatically activated valve interface (Valco Instruments Co., Inc., Houston, Texas, USA). Selected fractions were transferred to a Hewlett Packard 5890 Series II GC via a 350 µl loop interface and a short length of deactivated silica capillary. The speed of concurrent solvent evaporation optimised by venting through an early solvent vapour exit prior to the pre-column and analytical column. Following solvent evaporation the solvent exit was closed. A Hewlett Packard 5921A Atomic Emission Detector (AED) was used for multi-element detection. Transfer of the eluate from the GC was via a heated transfer line (320°C) into the cavity of the AED, which houses a microwave induced helium plasma (MIP).

RESULTS AND DISCUSSION

The LC solvent program developed, provided the initial elution of essentially all polycyclic aromatic hydrocarbons as a single unresolved peak 4 mins into the analysis. Polarity differences appear to be the major basis for class separation between the other classes of PAC present in the samples. Alkylated PAH species such as methyl chrysenes elute with the parent PAH compounds and require a less polar mobile phase to separate them from the bulk of components, therefore using a LC mobile phase of 15% DCM and 85% pentane, the parent and alkylated PAH species could be separated into distinct ring size fractions, and the ring size fractions produced can then be transferred directly to MS. Whilst the LC stage provides much improved PAC type selectivity the separation into particular groups eg parent, heterocycles, nitrated, oxygenated etc, is not totally complete since there is some polartity overlap of species in different groups. For example, large parent PAH compounds such as coronene, have similar retention characteristics as 2 ring nitro-PAC species.

Separation /Identification of Alkylated Polycyclic Aromatic Hydrocarbons: For the urban air particulate extract using LC-GC-MS, alkylated pyrene and chrysene compounds were found to make up the majority of alkylated substituted PAH in the air particulate, in the form of methyl/dimethyl/ethyl compounds. Using a 15% DCM and 85% pentane a ring size fraction of parent and alkylated PAH corresponding to the benz[a]anthracene/chrysene group was transferred to the MS. This separation is of particular interest in view of the marked difference in carcinogenic activity of methyl chrysenes.

Separation/Identification of Nitro-Polycyclic Aromatic Compounds: Most nitro-PAC produce an abundant molecular ion peak (M^+) and several characteristic fragments: $(M\text{-}NO)^+$ and $(M\text{-}NO_2)^+$. The fractions analysed were examined for the presence of mononitro-, dinitro-, methyl-nitro, and dimethyl-nitro PAC. 9-nitroanthracene (mass 223) and 1-nitropyrene (mass 247) were identified by comparison with GC retention times and mass chromatograms of reference compounds. An isomer of nitroanthracene was also identified. Derivatives of nitrated PAC (eg. alkyl or hydroxyl) are more difficult to identify via mass spectrometry, since they fragment differently from the mono-substituted nitro-PAC compound. No compounds of this type were identified have been identified to date by this technique, however attempts are continuing to develop a LC-GC-MS procedure for this analysis. Nitrated PAC are considered to be the most carcinogenic group of PAC[3], and their formation and atmospheric reactions are little understood, although there is some evidence that some are formed as artifacts of sampling by reaction with atmospheric OH followed by NO_2 attack[16,17].

Separation/Identification of Oxy-Polycyclic Aromatic Compounds: Oxygenated PAC are considered to be as carcinogenic as many of the parent PAH due to their similarity in structure and properties to the products of the PAH metabolic process. The metabolites formed can

decompose via triol intermediates which form adducts with DNA which then cause carcinogenic or mutagenic effects[3]. Detection of oxy-PACs was greatly simplified by their separation as a class from the nitro PACs. The identification of 9-anthraldehyde, 9-phenanthraldehyde and 9-fluorenone was confirmed by co-injection of standards. 9,10-anthracenedione (anthraquinone), plus its mono and di-substituted methyl derivatives which were all identified as being present in an urban air particulate extracts. A summary of the separation of urban air particulate extract by HPLC is shown in Table 1. Quinone-PAC are thought to be formed in the atmosphere from parent PAH by reaction with singlet molecular oxygen[18,19], formed either via a sensitization mechanism[20], or via ozonolysis[21].

Separation/Identification of Polycyclic Aromatic Heterocycles: The coupling of LC-GC separation with the Hewlett Packard atomic emission detector has vastly improved the ability of the instrument to speciate trace level heterocyclic PAC in complex hydrocarbon samples. Fractionation by LC enables interference from co-eluting hydrocarbons and more polar species to be eliminated, thus allowing sulphur (181 nm), nitrogen (174 nm) and oxygen (777 nm) PAC rich fractions to be transferred directly to the GC. For a typical diesel a series of substituted dibenzothiophenes, carbazoles and quinones were readily identified from the transfer of discrete LC fractions. For each element, the response was significantly enhanced due to increased sample concentration and the reduction in the formation of molecular species in the plasma. Indeed, the improved sensitivity of the detector operating within the LC-GC system, particularly for nitrogen has increased the reliability of elemental formulation.

CONCLUSIONS

Both LC-GC techniques provide an excellent technique for the rapid and much improved separation and identification of polycyclic aromatic compounds in fuel and environmental samples. Furthermore, the combination of elemental and compositional information from AED with molecular and structural information from MS offer a powerful approach to speciation of complex sample matrices. The increased compound-type selectivity in the primary separation improves the detection of very level species, by coupling on-line advantages with reduced sample complexity at the final separation stage. The limitations of systems are the individual detection capabilities of the mass spectrometer and atomic emission detector and the solubility of some of the most polar PACs in a solvent compatible with normal phase HPLC. However, both techniques so far have provided valuable insight into the source, formation and distribution of such compounds pre- and post combustion.

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Table 1. Separation of Urban Air Particulate Extract by HPLC.

Time Interval / min	Isolated Class / Compound(s)[a]
0 - 2.7	Solvent[b] Aliphatic compounds[b]
2.7 - 3.7	Polycyclic aromatic hydrocarbons[b] Alkylated PAH[b]
3.7 - 5.5	9-Nitroanthracene[b] Nitroanthracene isomer[b] 1-and 2-Nitronaphthalene 1-Nitropyrene[b]
5.5 - 6.5	2-Nitrofluorene[b]
11.5 - 14.0	9-Anthraldehyde[b] 9-Phenanthraldehyde[b] 2-Naphthaldehyde 9-Fluorenone[b]
14.0 - 17.0	9,10-Anthracenedione[b] Benzanthrone[b] 7,8 Benzoquinoline[b] benz[a]anthracene 7,12-dione[b] 2-Methyl-9,10-anthracenedione[b] 2-Fluorenealdehyde[b] 2-Nitro-9-fluorenone[b,c] 9-Anthracenone[b]
17.0 - 19.0	2-Methyl-1,4-naphthoquinone
19.0 - 21.0	1,4- Naphthoquinone[b]
24.0 - 25.0	Acenaphthenequinone[c]

[a]LC elution order based on reference standards
[b]Compounds identified in Leeds urban air particulate extract
[c]Compound only partially soluble in *n*-hexane.